

### **REMARKS**

Claims 1, 4, 9, and 11 have been amended herein. Support for the claims as amended can be found in the specification at page 3, third full paragraph; at page 4, first full paragraph; and at page 12, first and second full paragraphs. No new matter is added.

Claims 6, 7, and 12-21 were canceled in a previous paper.

Claims 1-5 and 8-11 remain in the application. Favorable reconsideration is respectfully requested.

#### **Rejection of Claims 1-5, and 8-11 Under §112, Second Paragraph:**

This rejection is believed to have been overcome by appropriate amendment to the claims. Specifically, the claims have been amended to recite that the streptokinase is expressed in the form of an inclusion body, and that the insoluble streptokinase contained in the inclusion bodies is enzymatically active upon solubilization of the inclusion body. This change in the positive recitation of the claims is believed to address the Examiner's concerns regarding using the adjective "enzymatically active" as applied directly to the inclusion body itself.

Applicants' intent with respect to the prior language of the claims was to require in the claims that the streptokinase expressed as an insoluble inclusion body is, in fact, "enzymatically active" when resolubilized, rather than being denatured and thus irreversibly inactivated. The claim language as amended is believed to clarify this point and thus overcome the rejection under §112, second paragraph. Withdrawal of the rejection is therefore respectfully requested.

Applicants wish to repeat here a point raised earlier: enzymes expressed in inclusion bodies are usually rendered irreversibly inactive because the enzyme becomes denatured in the inclusion body. See Applicants' prior response filed February 15, 2005 (in the paragraph spanning pages 7 and 8, and Exhibit A). In

short, one of ordinary skill in the art **cannot and will not** assume that an enzyme expressed as an inclusion body will be enzymatically active once the inclusion body is put back in solution. This is because most enzymes, when expressed as an inclusion body, are irreversibly denatured and thus enzymatically **inactive**, even when resolubilized. See Exhibit A of Applicants' response filed February 15, 2005.

In contrast, in the present invention the streptokinase is expressed in an inclusion body. That makes the enzyme far easier to isolate and purify. When the inclusion body is then resolubilized, the streptokinase is enzymatically active. Thus, the inclusion bodies themselves contain non-denatured, "enzymatically active" streptokinase.

**Rejection of Claims 1, 3 and 11 Under §102(b) in View of Pupo et al. (1999)**  
***Biotechnology Letters* 21:1119-1123**

The rejection of Claims 1, 3 and 11 is respectfully traversed because Pupo et al. clearly **do not** describe an expression construct which drives the expression of inclusion bodies comprising streptokinase (as required by Claims 1 and 3), nor do Pupo et al. describe a host transformed to contain such an expression construct (as required by Claim 11). Moreover, Pupo et al.'s insoluble fraction cannot have any endogenous streptokinase activity because, as per the Office's own definition, when an enzyme is insoluble it is inherently devoid of activity.

The first point, that Pupo et al. do not describe expressing streptokinase in an inclusion body, is made quite clear by the passage at page 1120, left-hand column, first full paragraph of Pupo et al.:

In this work we describe how the skc gene from *S. equisimilis* H46A can be expressed in *E. coli* W3110 as a soluble protein.... It was then possible to develop a simple procedure for the purification of the recominant SKC **while not including any solubilization** or refolding of this protein. (Emphasis added.)

Pupo et al. express an enzymatically-active streptokinase enzyme in *E. coli* without any solubilization of the resulting enzyme. By the Office's own definition, the enzyme produced by Pupo et al. must already be in solution. (See page 4 of the Office Action, "When an enzyme is insoluble it is inherently devoid of activity.") If Pupo's enzyme was insoluble, it would not have any activity and a solubilization step would be required. Therefore, Pupo et al. does not disclose expressing streptokinase in an inclusion body because Pupo's method does not include any solubilization step. (See the above-quoted passage.) In short, If Pupo's streptokinase was expressed as an inclusion body (which is required by the positive language of the present claims), Pupo's method would have to include a solubilization step. But Pupo et al. explicitly state that their method does not include a solubilization step.

The Office expresses puzzlement that Applicants keep raising the fact that any streptokinase activity found in Pupo's insoluble fraction is simply contamination from Pupo's soluble fraction. The point is this: Pupo's insoluble fraction does not contain any streptokinase activity of its own. Pupo's streptokinase activity is found entirely in the soluble fraction. The only reason there is any streptokinase activity in Pupo's insoluble fraction is due to contamination from the soluble fraction. Pupo himself says so at page 1122, left-hand column, first paragraph.

This issue is directly at the heart of the rejection: Pupo et al. explicitly state that their construct drives the expression of streptokinase "while not including any solubilization step." (Pupo et al., page 1120, left-hand column, first full paragraph.) By definition, then, the streptokinase formed in Pupo's approach is not in the form of an inclusion body because an inclusion body is insoluble. Pupo's insoluble fraction does not contain any endogenous streptokinase activity. As noted by Pupo himself, the presence of a small amount of streptokinase in his insoluble

fraction was contamination from the soluble fraction. The Office itself points out that when an enzyme is insoluble it is inherently devoid of activity. The point is that Pupo et al. **do not** express streptokinase in the form of an inclusion body.

The Office has taken an exactly opposite position, a position that is **flatly contradicted** both by the passage from Pupo et al. at page 1122, left-hand column, first paragraph, and the Office's own statement that an insoluble enzyme is inherently devoid of activity. Specifically, the Office states, at the top of page 4 of the Office Action that:

Pupo does not need to explicitly say that the "insoluble fraction" is insoluble SKC. Any one of ordinary skill in the art is fully aware that once one prepares a recombinant enzyme (in this case being SKC) **in inclusion bodies most** (if not all) of said enzyme is in insoluble form.

But Pupo et al. **do not** teach expressing streptokinase in an inclusion body. As noted above, Pupo et al. describe a method of making streptokinase **without any solubilization step**. As a consequence, Pupo's insoluble fraction **does not** contain any endogenous streptokinase. As Pupo himself stated, the small amount of streptokinase activity found in the insoluble fraction was contamination from the soluble fraction. Pupo et al., page 1122, left-hand column, first paragraph. The entire aim and goal of Pupo et al's work, as is clearly stated at page 1120, left-hand column, first full paragraph, was to make "a soluble protein... while not including any solubilization... of the protein." In short, Pupo et al. **do not** describe constructs that drive the expression of inclusion bodies that comprise streptokinase.

The Office Action also states, at the bottom of page 4 of the Office Action, that "Pupo is merely cited to indicate that SKC preparation in inclusion bodies has been done prior to this invention." In reply, Applicants note that Pupo et al. **do not** teach the expression of streptokinase in inclusion bodies. Moreover, the present claims **are not** directed solely to the expression of streptokinase in inclusion bodies. The present claims positively require a construct that drives the expression of

streptokinase in the form of an inclusion body and further that the streptokinase be enzymatically active when the protein is solubilized. This claimed invention simply is not taught, nor is it suggested, by Pupo et al. themselves nor any of the references cited by Pupo et al.

The Office also states, in the sentence spanning pages 4 and 5 of the Office Action, that "The method used to remove contamination from [the insoluble] SKC and what Pupo should have done to avoid contamination are totally irrelevant." Applicant agree entirely, but that was not the Applicants' prior argument. Applicants' earlier-submitted and current argument, which is critically relevant, is that Pupo's method drives the expresison of soluble streptokinase in the absence of a solubilization step. The only conclusion that can be drawn from Pupo's exact words are that his insoluble fraction is devoid of endogenous streptokinase activity. In contrast, the present claims require that an enzymatically active streptokinase be expressed in the form of an insoluble inclusion body.

Specifically regarding Claim 3, this claim requires the exact DNA sequence shown in Applicants' SEQ. ID. NO: 3. Pupo et al. simply do not disclose the required DNA sequence. Therefore, the Pupo et al. paper cannot anticipate Claim 3.

Because Pupo's insoluble fraction is devoid of any endogenous streptokinase activity, while all of the present claims are directed to constructs or methods that express streptokinase in the form of an insoluble inclusion body, Applicants submit that the rejection of Claims 1, 3, and 11 under §102(b) in view of Pupo et al. is untenable. Withdrawal of the rejection is respectfully requested.

**Rejection of Claims 2, 4-5 and 8-10 Under §103(a) over Pupo et al. (1999)  
*Biotechnology Letters* 21:1119-1123 in view of U.S. Patent No. 5,708,148 to  
Schmitz et al.**

This rejection is respectfully traversed because recombinant IGF-II, as taught in Schmitz et al., is a totally different protein than streptokinase. IGF-II is totally unrelated to streptokinase, has a totally different amino acid sequence than streptokinase, and has no logical link to streptokinase in any fashion. In short, a person of ordinary skill **would not** conclude, nor would be provided any reasonable expectation of success, that simply because IGF-II can be solubilized from an inclusion body, so too could streptokinase. The production of recombinant, enzymatically active enzymes simply cannot be generalized in this fashion.

The remarks provided above with respect to the Pupo et al. paper are repeated herein in full. Briefly recapping, Pupo et al. do not describe an expression construct which drives the expression of inclusion bodies comprising streptokinase, nor do Pupo et al. describe a host transformed to contain such an expression construct. And Pupo et al.'s insoluble fraction cannot have any endogenous streptokinase activity because, as per the Office's own definition, when an enzyme is insoluble it is inherently devoid of activity.

Streptokinase is a single chain polypeptide having 415 amino acid residues. See page 2, first full paragraph of the specification as filed and SEQ. ID. NO: 3. In stark contrast, IGF-II is only 67 amino acids long. See Schmitz et al., column 1, line 55, and SEQ. ID. NO: 1 of Schmitz et al. In short, the construct claimed by Applicants drives the expression of an inclusion body comprising streptokinase, a protein that is **519% larger** than the IGF-II described in Schmitz.

Applicants therefore submit that this rejection is clearly improper. There is **no reasonable likelihood of success**, based on Schmitz' ability to express IGF-II in an inclusion body and to recover the active IGF-II protein, that the same can be done with streptokinase, a protein that is more than 500% larger than IGF-II.

Proteins simply cannot be generalized in this fashion. The IGR-II protein described in Schmitz is vastly smaller than the streptokinase recited in Applicants' claims. The IGF-II protein is thus easier to solublize than is streptokinase due to its vastly smaller molecular weight as compared to the molecular weight of streptokinase.

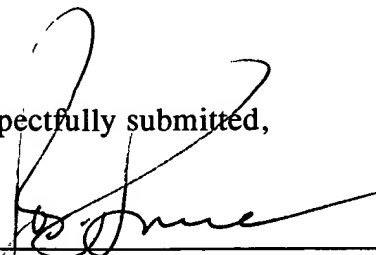
It is well known that proteins can and do have vastly different qualities, from egg whites to human hair. The simple fact that Schmitz can manipulate IGF-II into an inclusion body, and then solubilize the inclusion body to yield active IGF-II does not mean that the same can be done with streptokinase.

Applicants therefore respectfully submit that the rejection of Claims 2, 4-5 and 8-10 under 35 USC §103(a) is untenable. Withdrawal of the same is respectfully requested.

### CONCLUSION

Applicants submit that the application is now in condition for allowance. Early notification of such action is earnestly requested.

Respectfully submitted,

  
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